RESPONSE TO OFFICE ACTION

A. Status of the Claims

Claims 1, 8, 10-14, 17-22, 27, 31-33, 35, 37-41, 43-45, 49-52, and 54 were pending. Claims 8, 10-14, and 17-19 are canceled without prejudice. Claim 44 is canceled in view of the asserted objection. Applicants note that the currently pending claims all relate to use of dark lighting conditions in methods of cotton cell culture. Claims 1, 20-22, 27, 31-33, 35, 37-41, 43, 45, 49-52, and 54 are presented herein for reconsideration.

B. Objection to the Claims

Claim 44 was objected to as being duplicative of claim 37. Claim 44 has been canceled. In view of this, the objection to claim 44 is believed to be moot and its withdrawal is respectfully requested.

C. Rejections Under 35 U.S.C. § 103(a)

(1) Rejection of claim 1 in view of Finer and Rangan (1998)

(a) Finer does not demonstrate production of embryogenic callus from hypocotyl tissue

The Action maintains the rejection of claim 1 as obvious over Finer (Canada Patent 1,309,367) in view of Rangan (U.S. Patent 5,834,292). Applicants respectfully traverse.

The Action asserts that Finer teaches a method of producing pro-embryonic cotton cell masses that are capable of regenerating into mature embryos, and that callus formed from an explant such as a hypocotyl may be organized or non-organized, and may contain embryogenic callus and/or embryos (Finer, page 7, 5th paragraph). However, Applicants again note, as during the telephonic interview, that Finer, at page 7, 5th paragraph, explicitly states that when hypocotyls or cotyledons are used as explant sources, the callus that forms is **unorganized** *i.e.* lacking in pro-

embryonic cell masses, and thus is non-embryogenic. Although Finer apparently includes discussion of callus obtained from hypocotyls within a section of the Specification titled "Embryogenic cotton callus" at page 4, Applicants respectfully submit that mention is made there of such callus in order to contrast the "unorganized" nature of, for instance, hypocotyl-derived callus, with the "organized," *i.e.* embryogenic nature of callus derived from, for instance, somatic embryos, as illustrated in Finer Figs. 1-2 as well. This may be so that workers might distinguish between the different callus morphologies in order to select the most promising, and embryogenic, callus that would be proliferating from somatic embryos, as shown in Fig. 1 of Finer.

At page 5, 2nd full paragraph, the Action asserts that Finer teaches in Example 2 at page 16, that callus was induced from seedling hypocotyls. Applicants submit **that nowhere in Example 2 is it stated that such callus is embryogenic** (*i.e.* "organized"). That is, although Finer's Example 2 discusses callus induction from hypocotyl tissue, it does not state that such callus is embryogenic, while Finer's Examples 3-4, relating to suspension culture initiation and embryo development, do not utilize hypocotyl-derived tissues. In particular, the use of Medium 2 and Medium 3 in Example 2 of Finer demonstrates that Example 2 only covers initial steps of non-embryogenic callus induction, and **not embryogenesis**: Medium 2 (containing glucose and 2mg/l NAA (auxin); *i.e.* relatively high auxin) and Medium 3 (containing sucrose and 2mg/l NAA (auxin)) are apparently for initial non-embryogenic callus induction and/or subculture, (*e.g.* Finer, page 7, last paragraph). Thus, **Finer is not teaching conversion of cotton callus tissue, derived from hypocotyl tissue, from a non-embryogenic state to an embryogenic state**, since at page 7, 5th paragraph Finer states that embryogenic tissue can give rise to embryogenic callus, while non-embryogenic tissue (*e.g.* callus from hypocotyl) remains unorganized and non-embryogenic.

Finer apparently describes formation of embryos in his Medium #4, which is used in Examples 3-4. However, Applicants submit that the "100 g of callus tissue" that is "initiated" in Example 3 is **not** derived from hypocotyl tissue, especially in view of Finer's teachings at page 7, 5th paragraph, that embryogenic callus is to be formed from callus derived from somatic embryo explants, and not from hypocotyl explants. Thus, it would be a mischaracterization of Finer to conclude that the callus utilized in Example 3 is derived from hypocotyl tissue. Since M.P.E.P. 2143.03 states that all claim limitations must be considered, present claim 1 is not rendered obvious by Examples 2-4 of Finer, in that it recites use of hypocotyls as the tissue source. Further, Applicants again submit that this illustrates how methods for embryogenic cotton cell culture are distinct from methods for non-embryogenic, or organogenic, cell culture, as noted previously.

The Action further asserts at page 4, last two lines, that Finer "teaches that hypocotyls are the preferred explants for embryogenic cotton callus." Applicants respectfully submit that this is mistaken, especially in view of Finer at page 5, 3rd paragraph, which specifically states that somatic embryos are the preferred source for obtaining embryogenic callus; as well as in view of Finer, page 7, 5th paragraph, which states that hypocotyls yield unorganized (*i.e.* non-embryogenic) callus, as noted above.

Additionally, **the experiments of Finer Examples 2-4 are all performed with a 16:8 light/dark photoperiod**, as noted by Finer at page 15, bottom. Thus, even if embryogenic callus is being formed from hypocotyl tissue, which Applicants do not concede except *in arguendo*, such tissue is not being formed by dark culture conditions as in the presently claimed method. Since M.P.E.P. 2143.03 states that all claim limitations must be considered, claim 1 is not rendered obvious by Finer. Finally, Finer nowhere quantifies the efficiency of the methods described, while

the present Application demonstrates significant and unexpected improvements in tissue culture efficiency from use of dark culture conditions, as also discussed below.

(b) Neither Finer nor Rangan demonstrate production of embryogenic callus under dark conditions

Applicants also respectfully submit that **neither Finer nor Rangan actually demonstrate use of dark lighting conditions**, for instance as in claim 1, to allow for improved tissue culture efficiency. Finer provides **no actual results** that demonstrate obtention of embryogenic callus following growth under dark conditions, or improved efficiency in obtaining embryogenic callus by tissue culture performed under dark lighting conditions as presently claimed. For instance, at page 15, bottom, Finer states that the described media, *i.e.* in all of the subsequent examples, is utilized with a 16:8 hour light:dark photoperiod. At most, Finer provides only a most general teaching that culture may be performed in the dark, while explicitly stating that light is preferred (*e.g.* Finer, page 8, line 1-2).

Finer also provides no teachings regarding growth of different types of callus (*e.g.* non-embryogenic or embryogenic) in different light conditions (*e.g.* dark, or 16:8 light:dark photoperiod). Thus, Finer does not teach or suggest that, for instance, dark culture conditions are beneficial during growth on an embryogenesis-inducing medium in order to yield embryogenic callus, and the results discussed in section (c) below are clearly unexpected in view of the (lack of) teachings of Finer in this regard.

Finer further does not recognize or teach that dark culture conditions are a result-effective variable amenable to optimization as to the timing of a dark culture step- for instance whether during one or more of his steps (a)-(c), before or during embryogenesis. (M.P.E.P. 2144.05 (II) (B)). Finer's brief mention of possible use of dark conditions, at page 8, line 1, occurs during step (a) of his protocol, during initial (non-embryogenic) callus induction. Although Finer again

briefly mentions possible use of dark conditions during steps (b)- (c), such as during step (b) at page 9, 2nd paragraph, a practitioner would not understand that dark culture is particularly beneficial during induction of embryogenesis since: (1) use of a 16 hour photoperiod is generally preferred (*e.g.* page 9, 1st full paragraph); (2) a 16 hour photoperiod is used in all examples (page 15, bottom); (3) Finer's teachings regarding use of dark conditions in his steps (a)- (c) do not distinguish between non-embryogenic (*e.g.* initial callus induction) or embryogenic growth; and, as noted above, (4) no actual results regarding use of dark culture are given. In contrast, the present Specification for instance at page 6, lines 6-30, notes that transgenic non-embryogenic callus is to be cultured on an induction medium to promote formation of embryogenic callus, **in the dark**; and, for instance, Examples 2 and 9 of the present Specification specifically demonstrate enhanced efficiency for use of dark culture **during embryogenesis** (*e.g.* 1st section of Table 11).

Further, 11 years apparently passed between the priority dates of Finer and of the present Application (circa 1988- 1999), and Applicants submit that this also demonstrates that use of dark culture conditions was not an obvious parameter for a skilled artisan to alter in order to improve embryogenesis. Indeed, Rangan with a priority date of 1998 did not alter their lighting parameters to a dark culture regime (see below).

Regarding Rangan (U.S. 5,834,292), following the initial germination of cotton seed in the dark (*e.g.* column 6, line 65; or column 8, line 13), **subsequent tissue culture steps are stated to be carried out under 16:8 light:dark photoperiods** (*e.g.* Rangan, column 7, line 10; column 8, line 30; column 9, line 47; column 10, line 35; column 11, line 62; column 26, line 20). Thus, no use of dark lighting conditions in a method to produce transformed embryogenic cotton callus is demonstrated by Rangan, and the addition of Rangan to Finer, if anything, teaches away from use of dark conditions, instead further demonstrating that alternating light:dark photoperiods are to be

used. Applicants also respectfully submit that it is unclear why Rangan is combined with Finer regarding transformation, yet Rangan's teachings regarding use of a 16 hour photoperiod are being, apparently, arbitrarily disregarded, among the numerous potential experimental conditions, and combinations of experimental conditions, that might be utilized. Applicants respectfully submit that this is a *prima facie* demonstration of **hindsight reasoning** by the Action.

(c) The present application demonstrates unexpected results regarding embryogenesis under dark conditions

In contrast, the present Application provides ample experimental results demonstrating the effect of dark culture conditions, either alone or in combination with other experimental parameters, in improving tissue culture efficiency. For instance, Example 2 starting at page 25, and including Tables 2-3, demonstrates that dark conditions allow for a 2X-5X fold increase in the proportion of explant pieces that develop embryogenic callus, as compared to use of a 16 hour photoperiod. Examples 3-7 and 9 of the present Application further show how dark culture may be utilized in conjunction with other parameters in methods to enhance embryogenesis as well as to enhance the efficiency of later steps in producing embryos, maturing them, and germinating them to yield plantlets. Likewise, Example 9, beginning at page 33 illustrates the unexpected nature and degree of improvement described in the present Application. As discussed in Example 9, Protocol 1 from Example 1, utilized a 16/8 day/night cycle (*e.g.* Specification, page 25, line 15, and page 34, line 3), while Protocol 2 utilized incubation in the dark as described in Example 2, as well as other modified parameters of Examples 3, 5, 6, and 8. Table 11 demonstrates substantial improvement in frequency of embryogenic calli (*i.e.* increase of from 12% to 45% when comparing protocols 1 and 2). The possibility of such an **improvement in embryogenesis** via use of dark culture conditions is neither taught nor suggested by Finer in view of Rangan.. In view of these **unexpected results**, withdrawal of the rejection of claim 1 made in view of these references is respectfully requested.

(d) The use of Rangan represents a new rejection

Applicants also respectfully submit that the Rangan (1998) reference is used inconsistently and in a new manner in the pending Action. Specifically, in the previous Action, Rangan was combined with Finer in view of its asserted teachings regarding transformation of callus tissue. This is also stated at page 4 of the present Action, first two paragraphs. However, the present Action, as well as the discussion during the telephonic interview, also use Rangan to teach embryogenesis.

(e) Rangan is mischaracterized

The Action asserts that Rangan, at Example 26, describes transformation of cotton to produce plants. Applicants respectfully submit that this is mistaken. As noted during the interview, Rangan at Example 26 does not show that transformed plants were obtained, but rather that tissue from plants was transformed. This is made clear for instance at the bottom of the table in that example, which states "+ indicates that transformation of the tissue was performed" and not that plants were obtained. The method described in Example 18, stated to be used in Example 26, agrees with this, in that "plant segment" explants are transformed, **and callus is then selected for**, while possible further steps of regeneration of the transformed cells are not discussed. In any event, Examples 18 and 26 teach the use of a 16 hour photoperiod, unlike the present Example 1. Again, M.P.E.P. 2143.03 states that all limitations of a claim must be considered. Since claim 1 explicitly recites a method for use of dark conditions, and the present Application provides unexpected results, Rangan does not render claim 1 obvious when combined with Finer.

(2) Rejection of Claims 8, 10-12, 13 over Firoozabady in view of Davis, or Firoozabady, Davis, and Rangan

The Action asserts that Firoozabady (*In Vitro Cell Dev. Biol.* 299:166-172, 1993), in combination with Davis (*In Vitro* 9:295-398, 1974) renders claims 8 and 10-13 obvious. In view of

the cancellation of claims 8 and 10-13, the rejection is believed to be moot, and its withdrawal is respectfully requested.

(3) Rejection of Claims 14, 17, 18 over Firoozabady in view of Chi

The Action asserts that Firoozabady (*In Vitro Cell Dev. Biol.* 299:166-172, 1993), in combination with Chi (*Pl. Cell Rep.* 9:195-198, 1990) renders claims 14 and 17-18 obvious. In view of the cancellation of claims 14 and 17-18, the rejection is believed to be moot, and its withdrawal is respectfully requested.

(4) Rejection of Claim 19 over Firoozabady in view of Chi and Rangan

The Action asserts that Firoozabady (*In Vitro Cell Dev. Biol.* 299:166-172, 1993), in combination with Chi (*Pl. Cell Rep.* 9:195-198, 1990) and Rangan (U.S. 5,834,292) renders claim 19 obvious. In view of the cancellation of claim 19, the rejection is believed to be moot, and its withdrawal is respectfully requested.

(5) Rejection of claims 20-22 and 27 over Finer in view of Rangan, Davis, and Chi

The Action rejects claims 20-22 and 27 as obvious over Finer in view of Rangan, Davis, and Chi, cited above. Applicants respectfully traverse.

Applicants submit that claims 20-22 and 27 recite the use of dark culture conditions, and that M.P.E.P. 2143.03 states that all claim limitations must be considered. As noted for instance in section (1) above, Finer provides only the most cursory mention of the use of dark conditions, and **does not actually use them in any working examples**. Also as noted above, Finer does not recognize that timing of use of dark culture conditions is a result-effective variable that might be of interest for optimization (M.P.E.P. 2144.05 (II) (B)). In contrast, the present Application demonstrates an otherwise unexpected improvement in embryogenesis and overall efficiency of obtaining embryogenic callus and eventually transformed cotton plants, when dark culture

conditions are used (*e.g.* see Examples 2, 3, and 9). In view of the described **unexpected results**, withdrawal of the rejection is respectfully requested.

(6) Rejection of claims 31-33 and 35 over Finer in view of Rangan, Davis, Chi, and Firoozabady

The Action rejects claims 31-33 and 35 as obvious over Finer in view of Rangan, Davis, Chi, cited above, and Firoozabady (*Pl. Molec. Biol.* 10:105-116, 1987). Applicants respectfully traverse.

Applicants submit that claims 31-33 and 35 recite the use of dark culture conditions. As noted for instance in section (1) above, Finer provides only the most cursory mention of the use of dark conditions, and **does not actually use them in any working examples**. Also as noted above, Finer does not recognize that dark culture conditions are a result-effective variable that might be of interest for optimization (M.P.E.P. 2144.05 (II) (B)). In contrast, the present Application demonstrates an otherwise unexpected improvement in embryogenesis and overall efficiency of obtaining transformed cotton plants, when dark culture conditions are used in conjunction with certain other experimental parameters (*e.g.* see Examples 2, 3, 6, and 9).

For instance, Examples 6 and 9 utilize a support matrix to enhance embryo maturation. Table 9 at page 31 gives results of experiments in which wrapping of culture plates and light/dark conditions are compared. As shown in Table 9, by far the most efficient embryo maturation and germination was seen under dark conditions. Example 9, beginning at page 33 is a comparison of protocols. Protocol 1 from Example 1, utilized a 16/8 day/night cycle (*e.g.* Specification, page 25, line 15, and page 34, line 3). Protocol 2 utilized incubation in the dark as described in Example 2, as well as other modified parameters of Examples 3, 5, 6, and 8. Table 11 demonstrates substantial improvement in frequency of embryogenic calli (*i.e.* increase of from 12% to 45% when comparing

protocols 1 and 2). Further, embryo maturation and the overall efficiency of the experiments, regarding the number of plantlets obtained from a given number of starting explants, is also clearly enhanced.

The enhancement of embryogenesis and culture efficiency shown for instance in Table 11 are **clearly unexpected**, being neither taught nor suggested by Finer, and none of the other applied references teach or demonstrate are applied in order to cure this defect. Even regarding Firoozabady, which the Action asserts at page 7 to describe callus culture under dark conditions, the Action notes that Firoozabady then states that **germination and development of embryos under high lighting conditions is preferred,** which directly contradicts the present Application, for instance as described in Examples 2, 7, and 9.

Further, claim 31 recites in part: "…under dark lighting conditions of 0 μEinsteins $m^{-2}sec^{-1}$…" while M.P.E.P. 2143.03 states that all claim limitations must be considered in judging the patentability of a claim. Since the results such as shown in Tables 2-3, and 11 are **entirely unexpected** in view of Finer in combination with other applied references, the references do not render these claims obvious, and the rejection is without basis. Withdrawal of rejections made in view of these references is respectfully requested.

Additionally, Applicants point out that the rejection apparently confuses distinct steps in tissue culture, *e.g.* co-culture/ transformation which are early steps asserted to be described by Firoozabady (1987), with embryo maturation, discussed in the present Specification. Applicants further submit that the Action at page 20 is mistaken in reasoning that Firoozabady's (1987) asserted teaching regarding use of filter paper to reduce bacterial overgrowth (*e.g.* during co-culture/transformation with *Agrobacteria*) renders the presently claimed invention obvious.

This is because, by the time that the presently claimed step of embryo maturation is occurring, **bacteria are not present**, having been earlier selected against by use of, for instance, carbenicillin, cefotaxime, and/or kanamycin (*e.g* Specification at page 25, lines 9-11). Firoozabady also discusses selection against bacteria (*e.g.* p.106, paragraph bridging left and right columns). Thus, the asserted motivation for use of, for instance, filter paper, is simply not present during an embryo maturation step.

At page 21 the Action asserts that it would have been obvious to use a support medium during embryo maturation since it could have been used during the "initiation and transformation step." Applicants respectfully submit that maturation is performed on a different medium than initiation or transformation, and thus there would be no reason to continue use of support matrices during multiple tissue transfers, since this would instead be considered costly or wasteful, in the absence of any motivation to use filter paper or other support, throughout multiple subsequent culture steps, which Firoozabady (1987) does not provide. Again, M.P.E.P. 2143.03 states that all limitations of a claim must be considered when determining patentability, and claim 31 recites that the support matrix is utilized during growth on embryo maturation medium. Thus, the rejection is mistaken and its withdrawal is respectfully requested.

(7) Rejection of claims 37-41, and 43-44 over Finer in view of Davis, Chi, Firoozabady (1987), and Rangan (1993)

The Action rejects claims 37-41 and 43-44 as obvious over Finer in view of Davis, Chi, Firoozabady (1987), and Rangan (U.S. Patent 5,244,802). Applicants respectfully traverse.

As noted in section (1) above, Finer provides only the most cursory mention of the use of dark conditions, and **does not actually use them in any working examples**. Also as noted above, Finer does not recognize that the timing (*i.e.* during which culture step(s)) of dark culture conditions is a result-effective variable that might be of interest for optimization (M.P.E.P. 2144.05 (II) (B)).

On the contrary, a 16 hour photoperiod is preferred, and no teachings distinguishing use of dark culture during, for instance, non-embryogenic growth, embryogenic growth, or embryo maturation, are provided. Further, the present Application demonstrates an otherwise unexpected improvement in embryogenesis and overall efficiency of obtaining transformed cotton plants, when dark culture conditions are used in conjunction with certain other experimental parameters such as recited in claims 37-41 and 43-44. Additionally, as noted in section (6) above, use of a support matrix during embryo maturation is simply not taught or suggested by Firoozabady (1987) or any other applied reference. Although Rangan 1993 is also applied, apparently for its use of casein hydrolysate, this does not cure the defect in Finer or Firoozabady (1987). In view of the discussion at least of sections (1) and (6) above, relating to dark culture conditions and support matrices during embryo maturation, the rejection is believed to be without basis, and its withdrawal is respectfully requested.

(8) Rejection of claims 45 and 49 over Finer in view of Rangan (1998), and Gould

The Action rejects claims 45 and 49 as obvious over Finer in view of Rangan (1998), cited above, and Gould (*Pl. Cell Rep.* 10:12-16, 1991). Applicants respectfully traverse.

Gould is apparently applied in view of teachings relating to wrapping of culture containers with laboratory film. Applicants again submit that the teachings of Gould, relating to organogenic cotton cell culture, would not routinely be applied to embryogenic cotton cell culture methods.

The Action further asserts that wrapping would be obvious in order to prevent evaporation and contamination. Applicants submit that selective agents such as cefotaxime or carbenicillin are already being employed to prevent contamination. Further, the Action provides no basis as to why prevention of evaporation would be a significant variable in the mind of a skilled practitioner. In contrast, Applicants submit that it is at least as likely that a practitioner would consider whether wrapping would interfere with gas exchange, or lead to hyperhydricity (water logging) of cultures. Thus, the asserted reasoning is without basis. Additionally, in view of the discussion in section (1)

above, Gould does not cure the defect in Finer regarding use of dark culture conditions. Since claims 45 and 49 recite use of dark conditions, and M.P.E.P. 2143.03 states that all limitations in a claim must be considered, Applicants respectfully request that the rejection be withdrawn, in view of the **unexpected results** relating to cotton cell culture under dark conditions that are described in the present Specification.

(9) Rejection of claims 50-52 and 54 over Finer in view of Davis, Chi, Rangan (1993), Firoozabady (1987), and Gould

The Action rejects claims 50-52 and 54 as obvious over Finer in view of Davis, Chi, Rangan (1993), Firoozabady (1987), and Gould. Applicants respectfully traverse.

As noted in section (1) above, Finer provides only the most cursory mention of the use of dark conditions, and **does not actually use them in any working examples**. Also as noted above, Finer does not recognize that timing of use of dark culture conditions is a result-effective variable that might be of interest for optimization (M.P.E.P. 2144.05 (II) (B)). On the contrary, a 16 hour photoperiod is preferred by Finer, and use of dark culture during specifically any of steps (a)- (c) is not discussed. Further, the present Application demonstrates an otherwise unexpected improvement in embryogenesis and overall efficiency of obtaining transformed cotton plants, when dark culture conditions are used in conjunction with certain other experimental parameters.

Additionally, as noted in section (6) above, use of a support matrix during embryo maturation is simply not taught or suggested by Firoozabady (1987) or any other applied reference. Although Rangan 1993 is also applied, apparently for its use of casein hydrolysate, this does not cure the defect in Finer or Firoozabady (1987). Gould is also applied apparently regarding wrapping of cultures. However, in view of the discussion of section (8) above, Applicants submit that Gould is not properly applied. Further, Gould also does not cure the defect in Finer.

Thus, in view of the discussion at least of sections (1), (6) and (8) above, for instance relating to dark culture conditions and support matrices during embryo maturation, as well as the **unexpected results** described in the Specification, the rejection is believed to be without basis, and its withdrawal is respectfully requested.

D. Conclusion

In view of the above, it is submitted that all of the rejections to the claims have been overcome, and the case is in condition for allowance. The Examiner is invited to contact the undersigned at (214) 259-0932 with any questions, comments, or suggestions relating to the referenced patent application.

Respectfully submitted,

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